Food-Grade Emulsions

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High-Internal-Phase Pickering Emulsions Stabilized Solely by Peanut-Protein-Isolate Microgel Particles with Multiple Potential Applications

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Abstract: *High-internal-phase Pickering emulsions have various applications in materials science. However, the biocompatibility and biodegradability of inorganic or synthetic stabilizers limit their applications. Herein, we describe high-internal-phase Pickering emulsions with 87% edible oil or 88% n-hexane in water stabilized by peanut-protein-isolate microgel particles. These dispersed phase fractions are the highest in all known food-grade Pickering emulsions. The protein-based microgel particles are in different aggregate states depending on the pH value. The emulsions can be utilized for multiple potential applications simply by changing the internal-phase composition. A substitute for partially hydrogenated vegetable oils is obtained when the internal phase is an edible oil. If the internal phase is n-hexane, the emulsion can be used as a template to produce porous materials, which are advantageous for tissue engineering.*

High-internal-phase emulsions (HIPEs) are commonly referred to as superconcentrated emulsions with a minimum internal-phase volume fraction of 0.74.[1] Their wide applicability in pharmaceutical formulations, tissue engineering, and food products has received considerable attentions.[2] HIPEs contain an internal phase, a continuous phase, and necessary stabilizers to keep the system kinetically stable. In most cases, HIPEs are stabilized by large amounts of surfactants (5–50 wt%).[3] Some surfactants, such as carboxymethylcellulose and polysorbate-80, however, are subject to concerns, which may limit the application of HIPEs.[4] Besides the conventional emulsions mentioned above, so-called Pickering emulsions[5] stabilized by solid particles are usually more stable. Many surface-active particles adsorb irreversibly to the oil-water interface, thereby lowering the total free energy. In addition, a layer of adsorbed particles envelops droplets, thus endowing the emulsion with high stability to coalescence and Ostwald ripening.[6] High-internal-phase Pickering emulsions (HIPPEs) have been used as templates for the preparation of porous materials, in which the particles form the walls, which can result in a variety of applications.[1,7]

In general, many Pickering emulsions used currently have a relatively low dispersed phase fraction.[8] Nevertheless, HIPPEs have also been reported. Akartuna et al.[9] prepared HIPPEs with internal-phase volume fractions between 72% and 78%, but the systems required high particle concentrations (35 vol%). Zhou et al.[10] produced both methyl myristate-in-water and soybean oil-in-water(o/w) emulsions with high (80%) internal-phase fractions by using hydroxyapatite nanoparticles as emulsifiers and to further prepare porous materials; however, the route seems to be tedious and time-consuming (more than 7 days). Arditty et al.[11] reported HIPPEs with silane-modified silica particles that reached an internal-phase content of 90%. Ikem et al.[7b] and Li et al.[7c] also reported that by the use of inorganic particles or synthetic microgel particles as emulsifiers, stable HIPPEs with an internal-phase content above 90% could be prepared. Such HIPPEs are a good template for producing highly porous materials. Unfortunately the biosecurity, biocompatibility and biodegradability of these particles greatly limit their pharmaceutical applications.[12] On the other hand, Pickering emulsions are also important in the food industry,[13] which have a great demand for natural rather than chemosynthetic particles.

There is a growing interest in natural food-grade Pickering emulsions owing to their long shelf life and their ability to deliver functionally active substances,[13,14] and a few studies have been concerned with HIPPEs. Tan et al.[15] prepared HIPPEs stabilized by gelatin particles with an internal-phase of 80%. Zeng et al.[16] reported an antioxidant HIPPE stabilized by gliadin/chitosan hybrid particles with an internal phase up to 83%. However, none of the particles in the aforementioned studies were fabricated without using organic solvents (acetone, ethyl alcohol, or glutaraldehyde) or other reagents. The incorporation of organic solvents or residual reagents is a big concern when claims are made that the HIPPEs can be applied in the food industry. Capron and Cathala[6a] also reported high-internal-phase emulsions stabilized by cellulose nanocrystals, but the oil used was not edible, thus impeding application in the food industry. The clean-label HIPPEs applicable in the food industry should be composed of food-grade materials, including both oils and emulsifiers. HIPPEs composed of food-grade particles, water, and edible oil have a margarine-like appearance and can be of similar composition to margarine formulations,[17] which makes food-grade HIPPEs good substitutes for margarine[16] containing partially hydrogenated oils (PHOs). Serious food safety hazards mean that PHOs have been forbidden by the FDA to be used in food production in the USA after 2018.[18]

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Herein, we report high-internal-phase emulsions stabilized solely by natural peanut-protein-isolate (PPI) microgel particles, in which the fraction of the internal phase, either an edible oil or *n*-hexane, can be as high as 0.87 and 0.88, respectively. The PPI microgel particles adsorb at the oil–water interface to form a close-packed layer around the droplets, and some excess PPI microgel particles dispersed in the continuous phase form a network, both leading to stabilization of the emulsion against to coalescence. The prepared emulsions could have multiple potential applications simply by changing the composition of the internal phase. When the internal phase is an edible oil, it is possible to obtain a material with high viscosity similar to that of margarine. If the internal phase is *n*-hexane, a protein-based porous material can be obtained after evaporating the oil and water in air. The 3D structure of this material can be tailored by simply changing the pH value of the aqueous phase. Other applications are also possible with the present HIPPEs.

The preparation of peanut-protein-isolate microgel particles from PPI molecules involves two steps: i) transglutaminase (TG) enzyme cross-linking and ii) gel crushing. In the first step, TG-catalyzed acyl-transfer reactions between γ-carboxamide groups or protein-bound glutaminyl residues of peptides and primary amines of lysine promote the formation of an entire PPI hydrogel.[19] The above-mentioned hydrogel was crushed into microgel particles by using a rotor–stator homogenizer followed by a high-pressure homogenizer.

The protein-based microgel particles were amphoteric and pH-sensitive. The isoelectric point of the PPI microgel particles (where the overall charge on the surface of the particles was zero owing to the equal positive charges provided by amino groups and negative charges provided by carboxylic groups) and the PPI suspension (without TG enzyme cross-linking) was around pH 4.0–4.5 (see Figure S1 in the Supporting Information). Below the isoelectric point, PPI microgel particles are positively charged owing to the protonation of amino groups. Above it, they are negatively charged owing to the deprotonation of carboxylic groups. This charge variation results from the balance between the charges of carboxylic and amino groups of the peanut protein as a function ofpH value.

The average diameter of PPI microgel particles varied between 200 and 300 nm (Figure 1 a). Visible aggregates in water were formed at pH 3.0 and pH 4.5, thus precluding determination of their size by light scattering. Optical microscopy images showed that the size of the aggregates in aqueous solution was around 10–30 μm at pH 3.0 and 10–60 μm at pH 4.5 (see Figure S2). When the pH value was increased above the isoelectric point, the hydrophilicity of the PPI increased, which resulted in swelling of the PPI microgel particles (Figure 1 a). The three-phase contact angle of a water droplet on a substrate composed of PPI microgel particles in oil reached the highest value (156° for edible oil and 132° for *n*-hexane) at pH 4.5 (around the isoelectric point), implying the PPI microgel particles were at their most hydrophobic (Figure 1 b). This contact angle decreased either side of the isoelectric point, meaning that the hydrophobicity of the PPI microgel particles decreased at pH values both below and beyond the isoelectric point.

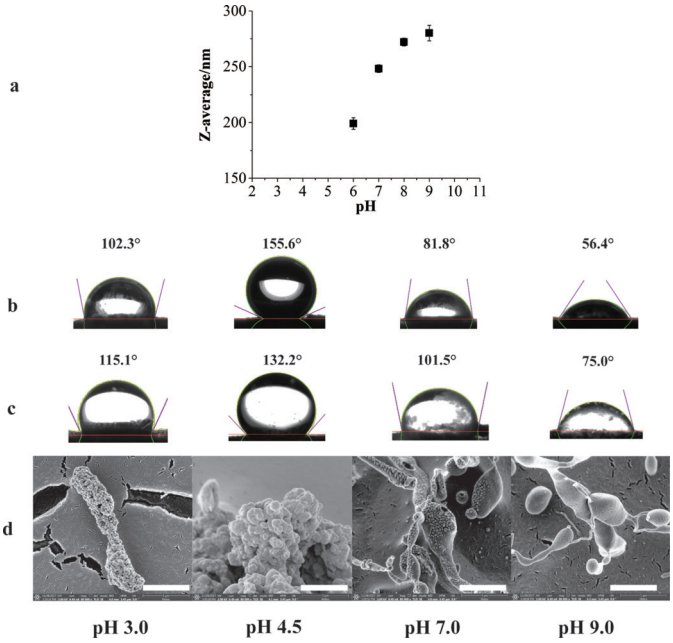

Figure 1. Characterization of PPI microgel particles: a) *Z*-average diameter at different pH values; b) three-phase contact angle of a water droplet on a substrate composed of particles in cold-pressed peanut oil at different pH values; c) three-phase contact angle of a water droplet on a substrate composed of particles in *n*-hexane at different pH values; d) cryo-SEM images of particles (1 wt%) in water at different pH values (scale bar: 1 μm).

The detailed morphology of PPI microgel particles was characterized by cryo-SEM (Figure 1 d). The particles were spherical with diameters of 40–150 nm, but could be in different aggregate states, and the extent of aggregation of PPI microgel particles depended on the pH value. The relatively large aggregates observed at a pH value of around 4.5 are associated with zero zeta potential, at which particles are most hydrophobic owing to a reduction in the electrostatic repulsion between them and to van der Waals attraction. As the pH value was lowered to 3.0, PPI microgel particles became positively charged, which resulted in stretching of the aggregates. As seen in the cryo-SEM image, the aggregates were composed of spherical particles, which aligned in strips. Above the isoelectric point, the aggregates were also linear in shape. Some ellipsoidal structures (major diameter: ca. 1 μm) could also be found. At pH 9.0, all particles were fused, and no individual particles could be found. TEM results were in accordance with the findings above (see Figure S3). However, the PPI microgel particles were smaller because they shrinked during the drying step in the preparation of the TEM sample.

Emulsions stabilized by peanut-protein-isolate microgel particles were prepared by mixing the oil phase (cold-pressed peanut oil or *n*-hexane) and aqueous particle dispersion at different pH values. Emulsification was carried out using a rotor–stator homogenizer at 3000 rpm for 30 s. When commercial cold-pressed peanut oil was used as the internal phase, a stable HIPPE with an internal-phase fraction of 0.85 could only be obtained at pH 3.0 and 9.0 (Figure 2 a,b), but not at pH 4.5 or pH 7.0. Both emulsions had a cream-like,

homogeneous appearance. From the cryo-SEM images, we can see that the oil droplets were trapped by a 3D structure formed by PPI microgel particles in the continuous phase (Figure 2c,f), which also provided steric hindrance to the coalescence of droplets. At pH 3.0, the droplet size was larger (5–50 μm; Figure 2c,e) than that at pH 9.0 (1–20 μm; Figure 2d,f)as PPI microgel particles in the aqueous phase

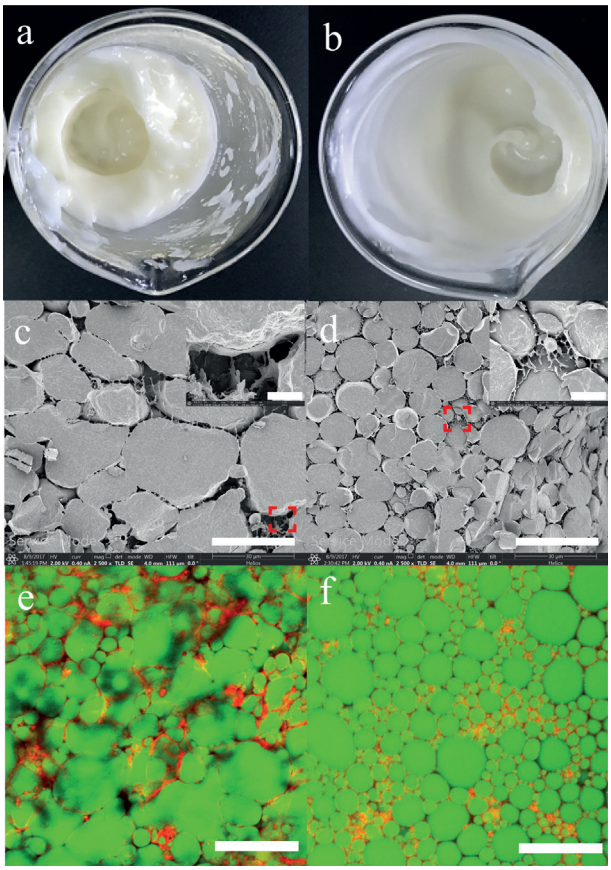

Figure 2. a,b) Digital photographs, c,d) cryo-SEM images, and e,f) CLSM images of HIPPEs with 85% cold-pressed peanut oil as the internal phase stabilized by PPI microgel particles (1.5 wt%). Insets in (c) and (d) are enlarged images of the area within the red boxes. The HIPPE in (a), (c), and (e) was obtained at pH 3.0, and the HIPPE in (b), (d), and (f) was obtained at pH 9.0. In (e) and (f), the oil phase is shown in green and the PPI microgel particles in red. Scale bars: 30 μm for (c–f) and 4 μm for insets in (c) and (d).

before emulsification were aggregated (see Figure S3a). Thus, aggregates of large "particle" size stabilized larger droplets. The smaller droplets formed during emulsification were subject to limited coalescence after the agitation stopped.[11] On the other hand, the diameter of the PPI microgel particles at pH 9.0 was around 270 nm (Figure 1a), and a larger interfacial area could be coated by them, thus leading to a smaller droplet size.

The other big difference is that the droplets were deformed at pH 3 (Figure 2c,e) but mostly spherical at pH 9 (Figure 2d,f). This difference should have an impact on the consistency and viscosity of the emulsions. The deformation may due to the fact that when the number of PPI microgel particles is insufficient to coat all the droplets, some PPI microgel particles may be shared by adjacent droplets in a bridging monolayer conformation.[20] This behavior leads to flocculation of the emulsion droplets without coalescence. HIPPEs with 85% (w/w) edible oil as the internal phase cannot be obtained at pH 4.5 and 7.0, which made the HIPPEs pH-responsive. The pH response of the emulsions may be divided into two scenarios. At pH 3.0, the striplike aggregates can attach to the interface and form a viscoelastic film to trap the oil droplets. At pH 4.5, the aggregates are the most hydrophobic (Figure 1b), and the oil/water (o/w) interface tends to bend towards the water phase. Besides, the big aggregates are too large to fully cover the huge specific surface area of an o/w HIPPE, so a water-in-oil (o/w) emulsion (with 15% internal phase) with a lower specific surface area was obtained (see Figure S4). At pH 7, an o/w emulsion with a high internal-phase fraction could be formed, but with excess coalesced oil surrounding the HIPPE (see Figure S4). At pH 9, the particles were swollen, deformed, and fused into an elastic interfacial film, which covered the oil droplets.[15,21] The upper limit of the internal-phase fraction within the emulsion was also established at pH 9.0. A HIPPE containing an oil-phase fraction of 0.87 could be reached. To the best of our knowledge, this is the highest reported value in all known food-grade Pickering emulsions.

PPI microgel particles at pH 3.0 and 9.0 were also used to stabilize HIPPEs with 85% *n*-hexane as the internal phase. The appearances of these emulsions are shown in Figure 3a,b. The macroscopic shapes can be readily tailored. The PPI microgel particle-stabilized emulsions at pH 3.0 and 9.0 were also characterized by cryo-SEM and CLSM (Figure 3c–f). In both emulsions, a dense layer of the PPI microgel particles can be observed (see insets in Figure 3c,d). The layer of particles surrounding the deformed droplets ensures the emulsion is mechanically stable.[7b] The oil and water of both emulsions were allowed to evaporate overnight in air. Evaporation resulted in a large number of pores in which the oil was located, with the adsorbed and excess PPI microgel particles forming the walls.[7c] From SEM images (Figure 3g,h), we can see that porous materials were obtained from both emulsions. The pore size is a little smaller than the precursor droplet size (see Figure S5), since PPI microgel particles were fused and formed uniform walls during solvent evaporation processing (see Figure S6). Furthermore, the thickness of the cell wall could be tailored by changing the concentration of the PPI microgel particles (see Figure S7). At low particle concentrations, the wall of the porous material shrank and was fragile (see Figure S7a,b, 0.83 and 1.17 wt%), and the pore structures almost collapsed. By increasing the concentration of particles, porous materials with relatively uniform walls could be obtained (see Figure S7a,b, 1.50 and 1.83 wt%). The upper limit of the internal-phase volume was also tested with *n*-hexane. PPI microgel particles at pH 9.0 can stabilize a HIPPE with 88% internal phase. These porous materials should have great potential as catalyst supports, in encapsulation applications, and as scaffolds for tissue engineering. With the great advantages of a natural product,

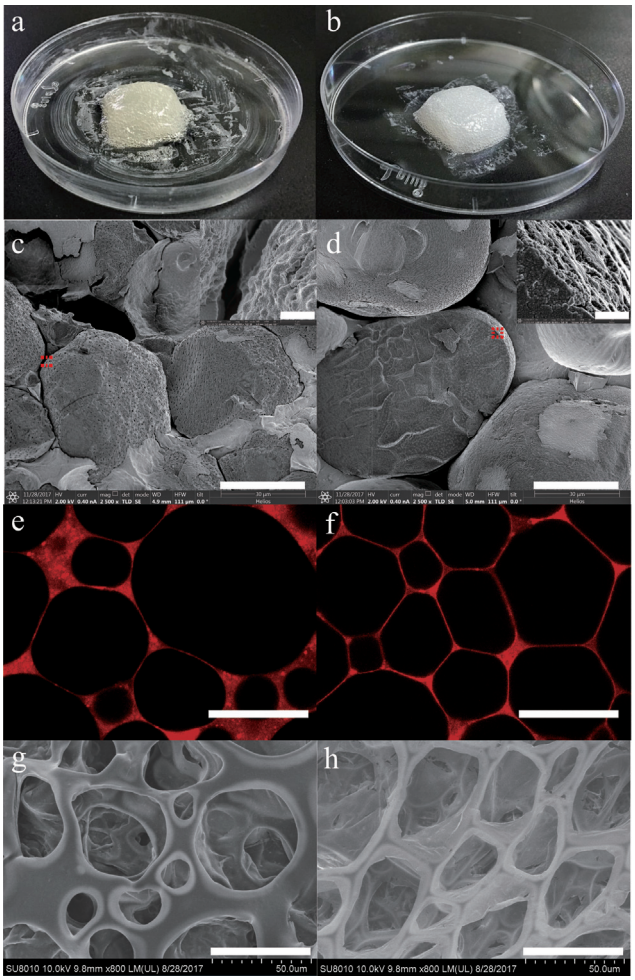

Figure 3. a,b) Digital photographs, c,d) cryo-SEM images, and e,f) CLSM images of HIPPEs with 85% *n*-hexane as the internal phase stabilized by PPI microgel particles (1.5 wt%); g,h) SEM images of porous materials prepared from the HIPPE templates. Insets in (c) and (d) are enlarged images of the area within the red box. The HIPPE in (a), (c), and (e) was obtained at pH 3.0 and transformed into the porous material in (g), whereas the HIPPE in (b), (d), and (f) was obtained at pH 9.0 and transformed into the porous material in (h). In (e) and (f), red corresponds to the particles. Scale bar: 30 μm for (c) and (d), 50 μm for (e–h), and 1 μm for insets in (c) and (d).

biotoxicity would not be a problem in applications compared to previous studies.[7c,d]

In summary, we have presented a new natural protein-based material (peanut-protein-isolate microgel particles) for stabilizing o/w Pickering emulsions. The internal-phase fraction can reach 87% for an edible oil and 88% for *n*-hexane. Edible materials as substitutes for PHOs can be obtained when the internal phase is an edible oil. Natural, nontoxic porous materials can be produced by using these Pickering emulsions as a template. We believe that the present emulsions can be used in a variety of areas, especially those with high biosafety requirements, such as food and tissue engineering.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: colloids · edible materials · gels · Pickering emulsions · porous materials

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[1] R. Butler, C. M. Davies, A. I. Cooper, *Adv. Mater.* **2001**, *13*, 1459.
[2] D. Wu, F. Xu, B. Sun, R. Fu, H. He, K. Matyjaszewski, *Chem. Rev.* **2012**, *112*, 3959.
[3] G. Q. Sun, Z. F. Li, T. Ngai, *Angew. Chem. Int. Ed.* **2010**, *49*, 2163; *Angew. Chem.* **2010**, *122*, 2209.
[4] a) E. Viennois, D. Merlin, A. T. Gewirtz, B. Chassaing, *Cancer Res.* **2017**, *77*, 27; b) P. D. Cani, A. Everard, *Trends Endocrin. Met.* **2015**, *26*, 273; c) G. G. Kaplan, S. C. Ng, *Gastroenterology* **2017**, *152*, 313; d) B. Chassaing, O. Koren, J. K. Goodrich, A. C. Poole, S. Srinivasan, R. E. Ley, A. T. Gewirtz, *Nature* **2015**, *519*, 92.
[5] S. U. Pickering, *J. Chem. Soc. Trans.* **1907**, *91*, 2001.
[6] a) I. Capron, B. Cathala, *Biomacromolecules* **2013**, *14*, 291; b) B. P. Binks, *Curr. Opin. Colloid Interface Sci.* **2002**, *7*, 21; c) T. S. Horozov, B. P. Binks, *Angew. Chem. Int. Ed.* **2006**, *45*, 773; *Angew. Chem.* **2006**, *118*, 787.
[7] a) M. S. Silverstein, *Prog. Polym. Sci.* **2014**, *39*, 199; b) V. O. Ikem, A. Menner, A. Bismarck, *Angew. Chem. Int. Ed.* **2008**, *47*, 8277; *Angew. Chem.* **2008**, *120*, 8401; c) Z. Li, T. Ming, J. Wang, T. Ngai, *Angew. Chem. Int. Ed.* **2009**, *48*, 8490; *Angew. Chem.* **2009**, *121*, 8642; d) V. O. Ikem, A. Menner, T. S. Horozov, A. Bismarck, *Adv. Mater.* **2010**, *22*, 3588.
[8] J. Wu, G. H. Ma, *Small* **2016**, *12*, 4633.
[9] I. Akartuna, A. R. Studart, E. Tervoort, L. J. Gauckler, *Adv. Mater.* **2008**, *20*, 4714.
[10] S. Zhou, A. Bismarck, J. H. G. Steinke, *J. Mater. Chem.* **2012**, *22*, 18824–18829.
[11] S. Arditty, C. P. Whitby, B. P. Binks, V. Schmitt, F. Leal-Calderon, *Eur. Phys. J. E* **2003**, *11*, 273.
[12] A. Nel, T. Xia, L. Mädler, N. Li, *Science* **2006**, *311*, 622.
[13] C. Linke, S. Drusch, *Crit. Rev. Food Sci. Nutr.* **2018**, https://doi.org/10.1080/10408398.2017.1290578.
[14] a) M. N. Corstens, C. C. Berton-Carabin, R. de Vries, F. J. Troost, A. A. Masclee, K. Schroen, *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 2218; b) J. Xiao, Y. Li, Q. Huang, *Trends Food Sci. Technol.* **2016**, *55*, 48; c) Y. Tan, K. Xu, C. Niu, C. Liu, Y. Li, P. Wang, B. P. Binks, *Food Hydrocolloids* **2014**, *36*, 70.
[15] H. Tan, G. Sun, W. Lin, C. Mu, T. Ngai, *ACS Appl. Mater. Interfaces* **2014**, *6*, 13977.
[16] T. Zeng, Z. Wu, J. Zhu, S. Yin, C. Tang, L. Wu, X. Yang, *Food Chem.* **2017**, *231*, 122.
[17] Standard for fat spread and blended spreads. CODEX STAN 256–2007.

[18] a) R. Voelker, *JAMA J. Am. Med. Assoc.* **2015**, *314*, 443; b) D. Mozaffarian, M. F. Jacobson, J. S. Greenstein, *N. Engl. J. Med.* **2010**, *362*, 2037; c) K. D. Brownell, J. L. Pomeranz, *N. Engl. J. Med.* **2014**, *370*, 1773.
[19] V.-D. Truong, D. A. Clare, G. L. Catignani, H. E. Swaisgood, *J. Agric. Food Chem.* **2004**, *52*, 1170.
[20] a) N. P. Ashby, B. P. Binks, V. N. Paunov, *Chem. Commun.* **2004**, 436; b) M. N. Lee, H. K. Chan, A. Mohraz, *Langmuir* **2012**, *28*, 3085.
[21] B. Brugger, S. Rutten, K. H. Phan, M. Moller, W. Richtering, *Angew. Chem. Int. Ed.* **2009**, *48*, 3978; *Angew. Chem.* **2009**, *121*, 4038; S. Wiese, A. C. Spiess, W. Richtering, *Angew. Chem. Int. Ed.* **2013**, *52*, 576; *Angew. Chem.* **2013**, *125*, 604.

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